

### **Amendments to the Specification**

*Please delete the paragraph on page 12, lines 12-14 and replace it with the following paragraph:*

Figure 4A is a Coomassie blue stained gel of 6XHis (**SEQ ID NO: 39**)-tagged recombinant yeast (r-y Sir2p) and murine (r-m Sir2 $\alpha$ ) Sir2 proteins purified with Ni-NTA agarose under native conditions. Arrowheads indicate each full-length protein.

*Please delete the paragraphs on page 16, lines 1-7 and replace it with the following paragraphs:*

Figure 14a is the amino acid sequence of the core domains of ySir2p (**ySir2**; SEQ ID NO: 11), mSir2 $\alpha$  (SEQ ID NO: 12) and CobB (SEQ ID NO: 13) aligned and six highly conserved residues, indicated by arrowheads, were mutated to alanine.

Figure 14b shows the 6XHis (**SEQ ID NO: 39**) tagged versions of wild type ySir2p (wt) and the six mutant Sir2p (Thr-261, Gly-270, Iso-271, Arg-275, Asn-345, Asp-347) and a vector control (vector) expressed in *E. coli*, purified over a Nickel-NTA column and analyzed on a 7% polyacrylamide SDS gel to assess expression levels.

*Please delete the paragraph on page 70, lines 11-18 and replace it with the following paragraph:*

The yeast *SIR2* gene or the mSIR2 $\alpha$  full-length cDNA was cloned into pET28a vector (Novagen). BL21(DE3) and BL21(DE3)pLysS with an extra copy of arginine tRNA gene was transformed with the ySIR2 and mSIR2 $\alpha$  plasmids, respectively. Each transformed bacterial clone was induced in 1mM IPTG at 37°C for 1hr. The induced 6XHis (**SEQ ID NO: 39**)-tagged proteins were purified with Ni-NTA agarose under native condition (see Figure 4A). The N-terminal fragment of mSir2  $\alpha$  was prepared in the same way. The control elute was prepared from a bacterial clone carrying pET28a vector only. The recombinant proteins were aliquoted and kept at -70°C.

*Please delete the paragraph on page 72, lines 20-26 and replace it with the following paragraph:*

The yeast *SIR2* gene or the *mSIR2*  $\alpha$  full-length cDNA was cloned into pET28a vector (Novagen). BL21(DE3) and BL21(DE3)pLysS with an extra copy of arginine tRNA gene was transformed with the *ySIR2* and *mSIR2*  $\alpha$  plasmids, respectively. Each transformed bacterial clone was induced in 1mM IPTG at 37°C for 1hr. The induced 6XHis **(SEQ ID NO: 39)**-tagged proteins were purified with Ni-NTA agarose under native conditions. The control elute was prepared from a bacterial clone carrying pET28a vector only. The recombinant proteins were aliquoted and kept at -70°C.

*Please delete the paragraph on page 88, lines 6-17 and replace it with the following paragraph:*

A set of mutations in highly conserved residues of the core domain of *SIR2* were constructed by site-directed mutagenesis (Figure 17a) and cloned into vectors, along with the wild type, to allow expression of the recombinant proteins in *E. coli* or expression of single-copy genes from the native *SIR2* promoter in *S. cerevisiae*. These 6Xhis **(SEQ ID NO: 39)** tagged proteins were purified from *E. coli* by a Ni-NTA column (Figure 17b) and analyzed for the NAD-dependent histone H3 deacetylase activity in an assay with a di-acetylated H3 peptide (residues 1-20 acetylated on Lys9 and Lys14) and 1mM NAD. HPLC separation of the reaction products yields five peaks of which 1, 2, a portion of 3 and 4 are deacetylated species of peptide (Figure 15). In this deacetylase assay, mutants 345 and 347 were inactive, mutant 261 showed 17% of wild type activity, mutants 270 and 271 showed 80% and 36% wild type activity, respectively, and mutant 275 showed 67% wild type activity.